Review

Oxidative signaling in seed germination and dormancy

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Abbreviations: MC, moisture content; ROS, reactive oxygen species; DW, dry weight; ABA, abscissic acid; GA, gibberellin

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Reactive Oxygen Species (ROS) play a key role in various events of seed life. In orthodox seeds, ROS are produced from embryogenesis to germination, i.e., in metabolically active cells, but also in quiescent dry tissues during after ripening and storage, owing various mechanisms depending on the seed moisture content. Although ROS have been up to now widely considered as detrimental to seeds, recent advances in plant physiology signaling pathways has lead to reconsider their role. ROS accumulation can therefore be also beneficial for seed germination and seedling growth by regulating cellular growth, ensuring a protection against pathogens or controlling the cell redox status. ROS probably also act as a positive signal in seed dormancy release. They interact with abscisic acid and gibberellins transduction pathway and are likely to control numerous transcription factors and properties of specific protein through their carbonylation.

Introduction

Reactive oxygen species (ROS) derivate from the reduction of oxygen which gives rise to superoxide ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO\cdot$) and singlet oxygen ($^1O_2$). Several reviews have described the signaling roles of ROS in plants (see for example, refs. 1 and 2) and their role in plant growth and development is now well documented.[3] For example, hydrogen peroxide have been involved in programmed cell death (PCD),[4-6] somatic embryogenesis,[7] response to wounding,[8] root gravitropism[9] and ABA-mediated stomatal closure.[10,11] The roles of superoxide and other ROS in signaling pathways are so far less well described; however $O_2^{-\cdot}$ seems to play a role in cell death and plant defence response.[12-14] In seeds, ROS production has been considered for a long time as being very detrimental, since the works dealing with ROS were mainly focused on seed ageing or seed desiccation, two stressful situations which often lead to oxidative stress.[15] Numerous recent works have nevertheless brought new lines of evidence showing that the role of ROS in seeds is not as unfavourable as it

was considered previously. At the opposite, it now appears more and more clearly that ROS would play a key signaling role in the achievement of major events of seed life, such as germination or dormancy release. The aim of this review is to present the current knowledge about the signaling role of ROS in orthodox seed germination and dormancy. This is actually particularly relevant because recent progresses in proteomics and transcriptomics have allowed the seed scientists to considerably enlarge their knowledge of seed dormancy and germination mechanisms, but these formers have also to be considered as a part of a complex regulatory network in which ROS are key signaling actors.

ROS Production and Sensing in Seeds

One of the most remarkable features of orthodox seeds is that their moisture content and metabolism vary dramatically from development to the completion of germination. Seed moisture content (MC) is high at the early stages of seed development, i.e., during embryogenesis, a developmental stage which is accompanied by an intense metabolic activity resulting in cell division. During seed filling, i.e., accumulation of reserves within the embryo or its surrounding structures, a sufficient amount of water is necessary for enabling metabolite transport through vascular connexions from a production source. This stage is followed by a dramatic loss of water during the so-called desiccation or maturation drying phase which requires the implementation of cellular adaptative mechanisms, among which ROS scavenging plays a key role,[15] for allowing seed survival.[16] At shedding, orthodox seeds are therefore fully desiccated (seed moisture content is generally below 0.10 g H_2O g dry weight (DW)) and quiescent, with a metabolic activity almost at a standstill.[17] Subsequent seed rehydration then allows germination, providing that the environmental conditions are appropriate or that the seeds are not dormant.

These contrasted situations, with regards to the hydration of the tissues, have marked consequences in term of ROS production. In developing or germinating seeds, the active mitochondria is probably one of the major sources of ROS, generating superoxide, and subsequently H_2O_2.[18,19] Approximately 2–3% of the oxygen used by the mitochondria results into superoxide and hydrogen peroxide production.[20,21] Chloroplasts can also generate ROS in the beginning of seed development, but they rapidly become non functional. The peroxisomes produce $O_2^{-\cdot}$ and H_2O_2, and in seeds, glyoxysomes, a particular type of peroxisomes involved in mobilization of lipid

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reserves produce high amounts of hydrogen peroxide resulting from the activity of enzymes such as glycolate oxidase.[22] Finally, NADPH oxidases of the plasma membrane, which transfer electrons from cytoplasmic NADPH to oxygen, are also a major other source of superoxide radicals, which subsequently dismutate to H_2O_2.[23,24] In dry seeds, enzyme activities are hardly probable,[16] although the existence of hydrated pockets has been suggested in dry tissues thus possibly allowing some metabolic activity in restricted cellular areas.[25] Therefore, ROS production in dry seeds would probably result mainly from non enzymatic mechanisms, such as those of Amadori and Maillard[26,27] and lipid peroxidation.[28] Lipid peroxidation is even favored at very low MC.[28] Non enzymatic oxidative processes have been extensively studied in food science because oxidative processes in manufactured products are involved in their shelf life. For example, it has been demonstrated that glasses, which are also likely to exist in dry seeds, do not prevent oxygen diffusion and autoxidation of lipids.[29-31] For ROS to act as cellular messenger, seeds must be endowed with a ROS removing system that tightly regulates their concentration. The ROS-scavengers in seeds have already been described elsewhere[15] and are not discussed here.

The ROS signaling transduction pathway in plants, and therefore in seeds, from sensing to changes in gene expression, is not fully understood yet. Again, the changes in cell water content during seed life suggest that seeds may cope with several sensing mechanisms. In hydrated tissues, for example during germination, presence of free water and low cytoplasmic viscosity, would allow ROS to travel within the cell. Therefore, as suggested by Moller et al.,[32] short-lived ROS, such as $HO^\bullet$, would react with sensors closed to their production site, whereas long-lived ROS, such as H_2O_2, could reach targets far from their production site. The established mechanisms of ROS transduction pathway might therefore be available in these conditions. They include MAP kinase cascade activation, inhibition of phosphatases, activation of Ca^{2+} channels and Ca^{2+}-binding proteins[1,2] but none of them have been clearly investigated in seeds. Conversely, in dry quiescent seeds, the absence of free water and an elevated cytoplasm viscosity probably limit the diffusion of ROS. Two nonexclusive hypotheses may be proposed for explaining ROS sensing in the dry state. The first one suggests that ROS would be "sensed" and then participate to the regulation of cell signaling in the dry state. This implies that cells would contain restricted hydrated zones allowing some molecular mobility, which would in turn permit ROS to be involved in cell signaling in these areas. Secondly, ROS could accumulate during dry storage but would become actors of cell regulatory mechanisms only after seeds get imbibed. It has indeed already been demonstrated that hydration of seeds causes a release of free radicals from the trapped state.[33]

Seed Germination

Germination is the process which leads to the elongation of the embryonic axis from a seed, allowing subsequent seedling emergence.[17] It consists in hydration of the quiescent seed (imbibition, phase I of the full process), and in the achievement of many metabolic and molecular events during the so called germination *sensu stricto* phase which occurs at a constant seed MC (phase II). Completion of the germination *sensu stricto* is the critical step of germination because it requires the activation of a complex regulatory system which is controlled by intrinsic (i.e., dormancy) and extrinsic (i.e.,environmental conditions, such as temperature, oxygen and water availability) factors. Many reports have shown that the transition from a quiescent seed to a metabolically active organism is associated with ROS generation, suggesting that it is a widespread phenomenon. Production of hydrogen peroxide has been demonstrated at the early imbibition period of seeds of soybean,[21,34,35] radish,[36] maize,[37] sunflower,[38] wheat,[39] pea[40] and tomato[41] seeds. Nitric oxide (NO),[42,43] hydroxyl radicals[36] and superoxide radicals[34-36] also accumulate during the germination of seeds of various species. However, the intracellular sources of ROS production are poorly documented. Presumably, most of the ROS produced should originate from mitochondria, since resumption of respiration in imbibed seeds might lead to electron leakage and increased production of ROS. However, the putative role of NADPH oxidase during germination is not known yet but should require attention regarding the various roles of this enzyme in various developmental processes. For example, Sarath et al.[43] demonstrated recently that NADPH oxidase inhibition delayed germination of warm season grasses. Several hypotheses can be proposed to explain the beneficial role of ROS during germination.

Endosperm weakening. In seeds of some species, such as tobacco, tomato, pepper, Lepidium or Arabidopsis, germination is constraint by the micropylar endosperm, which covers the radicle tip.[44-46] Germination can proceed if the mechanical resistance imposed by the endosperm decreases to such a level that radicle can protrude through the weakened tissues. This endosperm weakening is under the regulation of abscissic acid (ABA) and gibberellic acid (GA)[44] and several hydrolases (mannanase, cellulase, glucanase) have been suspected to contribute to cell-wall loosening.[25,47] There is an increasing bundle of evidence suggesting that ROS would play a key role in this phenomenon and it has been proposed that they would be involved in cell wall loosening in growing tissues.[48-50] Hydroxyl radicals have been shown to be present in the cell walls of growing organs[50,51] and they can break down polysaccharides by an oxidative scission of backbone bonds.[52-54] Their production could result from NADPH oxidase activity and /or Fenton reaction.[48,55,56] Chen and Schopfer[57] also suggested that cell wall peroxidases would play a role in hydroxyl radical formation. Furthermore, it has also been proposed that ROS might control polar growth through their effect on calcium channels[55,58] and Schopfer et al.[59] and Kawano et al.[60] suggested that auxin might promote cell growth through $O_2^{\cdot-}$ production and the subsequent generation of hydroxyl radicals. Based on these properties, and on the role of ROS in some physiological processes, such as fruit softening,[61] it is tempting to propose ROS as being involved in endosperm weakening during germination. Morohashi[41] demonstrated that a peroxidase activity developed in the tomato endosperm cap prior to radicle emergence. In a recent study, Müller et al.[56] also proposed that germination of Lepidium seeds, which requires endosperm rupture, involves the cleavage of cell wall polymers by ROS. They showed that H_2O_2 reversed the inhibitory effect of ABA on endosperm rupture, underlining the cross talk between these two compounds.

Protection against pathogens. It is well known that ROS production in plants may be used as a weapon against pathogens.[23] ROS may be either directly toxic against pathogenic microorganisms[62] or trigger hypersensitive reaction and programmed cell death at sites attacked by pathogens.[4,24] Many diseases being soil borne, the

germination and seedling emergence are critical steps with regards to putative pathogen infection. Germinating seeds of lupine produce an oxidative burst when inoculated with an avirulent pathogen.[63] Therefore, the rise in intracellular ROS in germinating seeds might constitute a defense reaction against infection by microorganisms. Additionally, extracellular production of ROS, extruded from germinating seeds, might also play a role in limiting the spread of invading pathogens by inhibiting spore germination and bacterial growth.[64] This hypothesis needs however additional evidence for being properly addressed.

Redox regulation. Another attempt for explaining the possible role of ROS accumulation during germination was to study protein carbonylation, an irreversible oxidation process leading to a loss of function of the modified proteins. Although protein carbonylation is often associated with aging in animals, it was demonstrated to occur in high vigor germinating Arabidopsis seeds, yielding vigorous plants.[65] Interestingly carbonylation was not randomly distributed among the protein pool but directed against specific proteins, such as reserve proteins, including cruciferin subunits for example. Carbonylation of reserve proteins would help in their mobilization during germination by increasing their susceptibility to proteolytic cleavage.[65] Proteins involved in glycolysis/gluconeogenesis pathways also became carbonylated during germination which could provide reducing power through the pentose phosphate pathway. This former hypothesis is particularly relevant because germination have often been proposed to be regulated by the cellular redox status. Redox regulation mainly leads to an alteration in the activity of target proteins via thiol-disulfide exchange, and is controlled by cellular redox agents, such as thioredoxins or glutathione/glutaredoxin.[66,67] The involvement of thioredoxins in germination, particularly demonstrated in cereal seeds, begins to be well characterized. Thioredoxin h has been shown to facilitate the reduction of intramolecular disulfide bonds, which would in turn promotes the degradation of reserve proteins and the activation of various proteases.[68,69] During germination, ROS might have an effect on the level of the ROS scavengers thioredoxin or reduced glutathione, and thereby modulate the redox signaling of these compounds.[70] Alternatively, various transcription factors have been shown to sense ROS via the formation of disulfides involving thioredoxin and glutaredoxin.[67] However, as suggested by Foyer and Noctor,[3] the exact role of the redox sensing agents is often difficult to establish precisely because they are involved concurrently in redox signalisation cascades and defense against ROS.

Aleurone layer programmed cell death. ROS, especially H_2O_2, have also been implicated in aleurone programmed cell death (PCD) of cereal grains during germination and seedling establishment.[4,71] Aleurone layer of germinating cereal grains synthesizes and secretes hydrolytic enzymes for sustaining seedling growth before undergoing PCD.[72,73] Activation of lipid and respiratory metabolisms leads to ROS proliferation, which are mainly generated in the aleurone cell glyoxysomes and mitochondria.[74] This process is tightly regulated by the hormonal balance. GA promotes germinative and post-germinative processes, while ABA inhibits them.[73,75] Exogenous H_2O_2 induces cell death in GA- but not in ABA-treated cells, which is related to the differential ability of these tissues to scavenge H_2O_2. While the activity of ROS scavenging enzymes, such as CAT, APX and SOD, is down-regulated in GA-treated layers, it is maintained in ABA-treated cells,[4] which suggests that the capacity to cope with

ROS seems to be linked to PCD execution. It has been reported that a decline of glyoxysomal catalase precedes PCD in aleurone cells and may contribute to an increase in cellular oxidative stress.[4,74] ROS may then damage DNA, proteins and membrane lipids resulting in the loss of protein functions and membrane integrity. Other factors seem to regulate PCD in aleurone cells. Thus, blue and UV-A light accelerate PCD of GA-treated aleurone protoplasts but can not induce it in ABA-treated protoplasts.[76] NO donors, in contrast, delay the loss of CAT and SOD and may be an endogenous modulator of PCD in barley aleurone cells.[77] Interestingly, Kranner et al.[78] showed recently that PCD also occurs in whole seeds and that the half-cell reduction potential of glutathione was involved in its initiation thus underlying the role of oxidative signaling in this process.

Finally there is still a domain to be explored in future studies dealing with germination and ROS, which concerns the direct effect of these compounds on gene expression. Changes in gene expression during germination[79-81] or dormancy release[82-84] are actually becoming more documented. The effects of ROS, and more particularly H_2O_2 on transcriptome have also been widely studied.[85,86] However, up to date, there is no information available establishing a direct link between the changes in ROS content and gene expression during germination.

Seed Dormancy

Seed dormancy may be defined as the property of a seed that prevents its germination in apparently favorable conditions.[17,46,87] It is a poorly understood phenomenon, influenced by the genetic background of the species, the environmental conditions and the balance of ABA and GA. Dormancy may be related to the embryo itself or to its surrounding structures (i.e., seed coat) which leads to distinguish several classes of dormancy.[46] As the aim of this review is to focus on the signaling role of ROS, we will only present results dealing about their putative involvement in embryo (i.e., physiological) dormancy. Depending on the species, release of dormancy may mainly occur during after-ripening (storage in dry conditions) or during cold or warm stratification (imbibition at low or warm temperatures).[17,46,87-90] In both cases dormancy alleviation is associated with a widening of the environmental conditions allowing seed germination. Additionally, several compounds, often belonging to plant hormones, are known to allow the germination of dormant seeds; they include for example gibberellins, ethylene or cyanide.

Seed after-ripening. Seed after-ripening occurs at such low seed moisture contents (MC), generally less than $0.10 \, g \, H_2O/g \, DW^{-1}$, that water is probably not available for biochemical reactions. However, under these drastic conditions, seeds undergo dormancy alleviation. Leubner-Metzger[25] proposed that local hydrated pockets within cells or tissues of the dormant seeds would allow changes in gene expression in the dry state. ROS been playing a role in cellular signaling, Bailly[15] suggested that these compounds could facilitate the shift from a dormant to a nondormant status in seeds. Indeed, it is known that ROS can accumulate during seed storage in the dry state, as previously mentioned.[28,91-93] Oracz et al.[94] and El-Maarouf-Bouteau et al.[95] demonstrated that there was a clear cut relationship between sunflower seed dormancy alleviation and accumulation of ROS and peroxidation products in cells of embryonic axes, thus suggesting that ROS might play a role of signal in dormancy alleviation. These authors showed that proteins were one of the targets of ROS, since a

pool of them became specifically carbonylated during after-ripening. Among this pool, some storage proteins became oxidized suggesting that breaking of dormancy in the dry state would be associated with a preparation toward storage protein mobilization. Interestingly, specific protein carbonylation also appeared when imbibed dormant sunflower seeds were treated by a dormancy release compound such as cyanide. Conversely, methylviologen, a ROS generating compound, alleviated dormancy and also triggered specific protein oxidation. This emphasizes the role of ROS and protein oxidation as a putative general mechanism of dormancy breaking, which will have to be assessed with dormant seeds of other species.

These former data are the first showing that ROS play a role in seed dormancy alleviation through at least protein oxidation. Because ROS are also known for being associated to gene expression, the possibility that they could act at genome level during after-ripening may be proposed. Modification of the genetic programme associated with the transition from a dormant to a nondormant state has been described in few reports. Apparent gene expression was detected during after-ripening in *Nicotiana tabacum*,[25] *Nicotiana plumbaginifolia*,[82] Arabidopsis,[83] barley[84] and sunflower.[95] During after-ripening, numerous genes are differentially accumulated in dormant and nondormant seeds. Many of the genes up-regulated in nondormant seeds are associated with protein synthesis, potentially controlling the completion of germination.[82,83] A proteomic analysis comparing dormant and after-ripened Arabidopsis seeds have shown a specific differential accumulation of proteins suggesting that proteins potentially involved in seed germination can accumulate during after-ripening.[96] Although these data show that transcriptional and translational events could be a component of dormancy loss process, they provide little evidence on how genes are acting and whether ROS act directly or indirectly on gene expression during dormancy alleviation. Furthermore, because many of the highly expressed genes in the dormant state are stress-related, it was proposed that ABA, stress and dormancy responses overlap at the transcriptome level.[83]

Hormones and the control of seed dormancy. Dormancy maintenance and release depend mainly on intrinsic balance of ABA and GA. While dormancy maintenance depends on high ABA/GA ratios, dormancy release involves increase of GA biosynthesis and ABA degradation resulting in low ABA/GA ratios.[83,97] GA has been described as an internal regulator involved in the induction of germination, whereas ABA is widely recognized as being the most important hormone involved in the establishment and maintenance of seed dormancy.[43,87,98] ABA, which imposes dormancy, originates from the seed itself during seed development and de novo ABA biosynthesis has been reported during imbibition of dormant seeds.[97-101] Mutants altered in hormone biosynthesis or hormone response/signal transduction allowed to better understand the role of each hormone and the interaction between the different hormone pathways (reviewed in ref. 44). Indeed, ABA deficiency during seed development is associated with the absence of primary dormancy and ABA overproduction is associated with enhanced dormancy. During after-ripening, associated with dormancy release, decreases in ABA sensitivity and concomitant increase of sensitivity to GA have been reported. There are also antagonistic effects of ABA and ethylene on dormancy and germination. Ethylene may promote germination by interfering with the ABA action on seed dormancy and/or maintenance of dormancy.[102] Ethylene is implicated in the promotion of germination of nondormant seeds of a wide range of species.[103-107] In some species, such as sunflower, ethylene can break seed dormancy.[103] Several components of the ethylene signal transduction have been identified and their signaling pathway has been characterized.[44,108] Ethylene receptors are normally blocked to repress ethylene response.[109] Upon ethylene binding, the receptors become active which alleviates the repression on ethylene signal transduction and allows ethylene responses.[110] Ethylene signal transduction mutant studies highlight the interaction between ABA and ethylene signaling suggesting that ethylene suppresses dormancy by inhibiting ABA action.[44]

There are therefore strong presumptions that interactions between ABA, GA, ethylene and other hormones like auxin or brassinosteroids and extensive cross-talk among their respective signaling pathways play a major role in seed dormancy and germination.[111] One may nevertheless wonder what is the nature and extent of the overlap and cross-talk in hormone responses, and how the switch from a dominant hormone signaling pathway to another occurs during the transition from seed dormancy to germination. Signal molecules such as ROS could be good candidates in this process since the interaction between hormones and ROS in other developmentally controlled processes in plants has been reported (reviewed in ref. 112).

Among the possible interplay between ROS and plant hormones, the relationship between H_2O_2 and ABA appears as the most probable. On the one hand, there exists evidence suggesting that hydrogen peroxide alleviates seed dormancy. Exogenous H_2O_2 stimulates the germination of dormant seeds of barley,[100,113,114] rice,[115] apple[116] and *Zinnia elegans*.[117] As early as 1975, Hendricks and Taylorson[118] showed that inhibition of catalase activity promoted the germination of dormant seeds of lettuce and pigweed. Oracz et al.[94] demonstrated that cyanide, a compound releasing sunflower seed dormancy, triggered ROS accumulation and protein oxidation. On the other hand, H_2O_2 seems to have a role in cellular response to ABA at the level of the gene expression and by regulating ion movements in guard cells.[10,119] Furthermore, in vitro biochemical studies revealed that H_2O_2 inactivates ABI1 and ABI2 type 2C protein phosphatase, enzymes that function in ABA signaling.[120,121] Treatment of dormant barley seeds with hydrogen peroxide results in a decrease in endogenous ABA level[100,114] and alleviation of apple embryo dormancy by cyanide induces a simultaneous increase in H_2O_2 level and decrease in ABA content.[116]

Ethylene and ROS signalling pathways seem also to share some common mechanisms. Stimulation of ethylene synthesis by environmental stresses, such as ozone, UV irradiation, and wounding, involve generation of reactive oxygen species.[122] Interactions among salicylic acid (SA), jasmonic acid (JA) and ethylene have been also reported and found to modulate responses to ROS (reviewed in ref. 123). Furthermore, it was shown that ethylene receptor ETR1 plays an important role in guard cell ROS signaling and stomatal closure.[124]

Finally, interaction between GA signaling and ROS was demonstrated in stem elongation.[125,126] Maya-Ampudia and Bernal-Lugo[127] showed that GA_3 modified the redox status of aleurone proteins during germination, a process which might be related to ROS accumulation.

These data give strong indications about the interaction between hormones and ROS but there is up to date few results clearly demonstrating their crosstalk in seed dormancy. However, there is a growing

evidence of the overlap of hormone signaling and ROS regulation in seed germination as described below. Furthermore, emerging evidence suggests that hormone signaling pathways regulated by ABA, SA, JA and ethylene, as well as ROS signaling pathways, play key roles in the crosstalk between biotic and abiotic stress signaling.[128] Whether hormones and ROS interact in seed dormancy release and during germination process is still unknown but highly credible.

NO, a putative key player in the control of seed dormancy. NO, a gaseous free radical that can be generated from nitrates, has also been proposed to be involved in seed dormancy alleviation. It was proposed that NO played a role as an endogenous regulator of germination in Arabidopsis and barley.[129] However, NO promoting germination can be blocked by sufficiently high concentrations of ABA.[129] Recently Bethke et al.[130] demonstrated that Arabidopsis aleurone cells responded to NO, with NO being upstream of GA in a signaling pathway leading to dormancy alleviation, by up-regulating *GA3ox1* and *GA3ox2*, two genes involved in the biosynthesis of active GA in Arabidopsis seeds. Other nitrogen-containing molecules such as azides and hydroxylamines stimulate germination of dormant seeds probably via their metabolism to NO.[118,131] The focal point of convergence of both NO and H_2O_2 could be the fact that azide and hydroxylamine are converted to NO in a reaction catalyzed by catalase in the presence of H_2O_2.[132] In addition, the NO-activated MAPK in tobacco can also be activated by H_2O_2.[133] NO and H_2O_2 are commonly present during various stresses as reported in bacterially-induced PCD in soybean and Arabidopsis.[134,135] Increase of ROS during dormancy release could induce NO production which can act in addition with H_2O_2 in the same MAPK signaling pathway.

Stratification and light. Seed dormancy is also broken, in many plant species, by cold stratification, a prechilling treatment of fully imbibed seeds. Mechanisms underlying the physiological changes during cold stratification are still unknown but seem to be tightly related to GA. In fact, cold and light responses are mediated, in part, by promoting GA biosynthesis via enhanced expression of GA3 oxidase in Arabidopsis (*AtGA3ox*).[136-140] Light has been described to stimulate germination and to terminate dormancy of many species.[141-143] In seeds with coat dormancy, it is thought that light and GA can both release (coat) dormancy and promote germination.[44,144-146] Exogenous nitrate can affect the requirement for light to promote Arabidopsis seed germination. Nitrate regime fed to the mother plant seems to influence the initial level of Arabidopsis seed dormancy[147] and germination,[148] probably by a decline in ABA content in imbibing seeds.[97,149]

Previous studies have also highlighted tight links between ROS, cold and light in other plant processes. It has been reported that light and particularly UVB radiation produce ROS such as superoxide, H_2O_2 and singlet oxygen.[150-154] A-H-Mackerness et al.[155] indicated that ROS play a pivotal role as secondary messengers in a number of UVB signal transduction pathways among key regulators. Indeed, ROS and plant hormones, SA, JA and ethylene have been shown to be key regulators of gene expression in response to UVB exposure.[122,155-157] In plant responses to temperature stress, increase in ROS has been implicated. H_2O_2 has been specifically involved in low temperature responses in plant.[158] As temperature and light are the major natural factors conditioning seed dormancy and germination, it has now to be determined if ROS is the signal linking environmental stimuli to the hormone signalisation.

Conclusions

Seed dormancy and germination are very complex phenomena which involve tightly controlled signaling pathways and molecular regulations. In addition to hormones, there are many signaling molecules such as ROS and NO that seem to play a role in this process, but whether these processes relies on an unique dominant signaling pathway or on the overlap of many is unknown. A putative model, based on the references cited in the text, in which hormones and ROS compete for seed dormancy release and germination might be particularly attractive (Fig. 1). We assume here that germination is accompanied by ROS release, that ABA inhibits ROS accumulation and that GA reverses this inhibition. The hypothesis shown (Fig. 1) postulates that ROS play a central role with hormones, and particularly ABA, in dormancy release and germination completion. In the dormant state, ABA signaling pathway is active and prevents germination. High amount of ABA may maintain high level of ROS scavenging enzymes leading to a low level of ROS during imbibition of dormant seeds. During after-ripening there is an accumulation of ROS by nonenzymatic ROS production. This accumulation might reduce ABA level and/or block ABA signalling, stimulate GA signaling, modify redox status and downstream events and alter protein function through oxidative modifications. Subsequent imibition of after-ripened seeds would therefore be associated with the completion of germination. Further experiments in this area, especially in the -omics science, are likely to be highly informative for getting a comprehensive view of germination and dormancy release controlling events.

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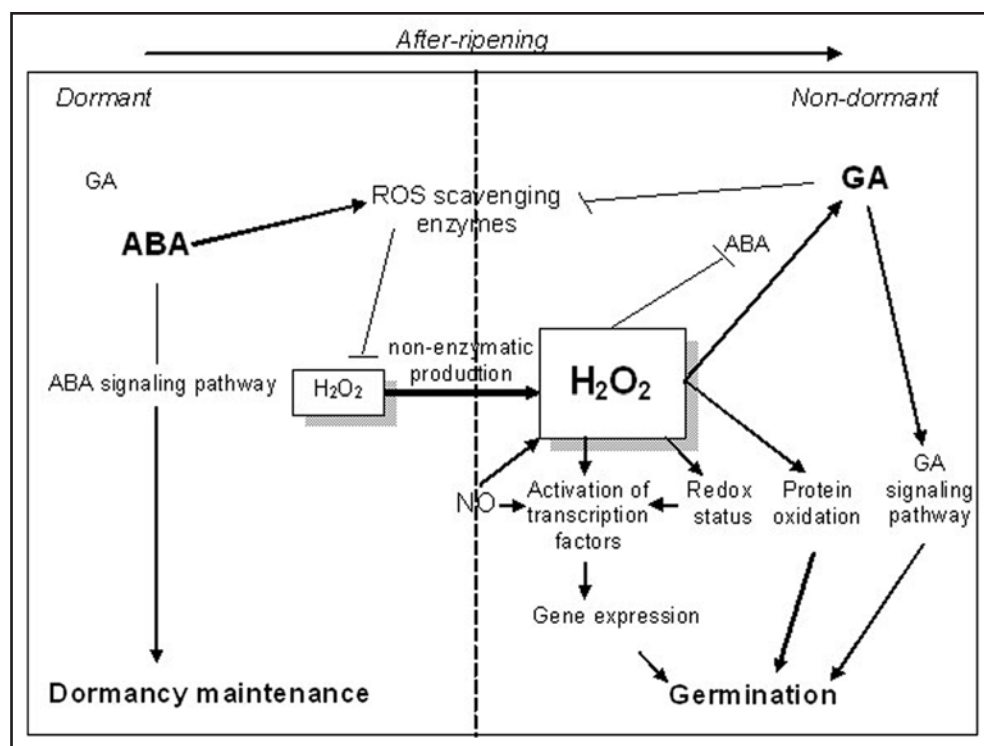
Figure 1. Hypothetical model proposing a central role of ROS in seed dormancy release and germination. In dormant state, high amount of ABA induces an active signaling pathway involved in dormancy maintenance. Lower ABA concentration in nondormant seeds would be associated with a higher level of ROS, presumably H_2O_2, which could in turn interfere with ABA and GA signaling pathways, modify redox status and induce protein carbonylation. NO may interfere with H_2O_2 and activate GA signaling. The size of the letters is proportional to the relative amount of the compounds (ABA, GA, H_2O_2).

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